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## MOLECULAR SYSTEMATICS OF THE NEOTROPICAL DIVING BEETLE GENUS *RUGOSUS* GARCÍA, 2001 (COLEOPTERA: DYTISCIDAE: COPELATINAE)

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### ABSTRACT

In recent years, several new genera have been erected in the subfamily Copelatinae, including the description of endemic taxa from Madagascar, New Guinea, South Africa, and Venezuela. Here we build upon a recent molecular phylogeny of this subfamily to investigate the phylogenetic placement of *Rugosus* García, 2001, a genus comprising two species from the Guiana Shield region of Venezuela. We sequenced one mitochondrial and one nuclear gene fragment of *Rugosus emarginatus* García, as well as a specimen of *Aglymbus lepriurii* Aubé from Guyana in order to reassess the phylogenetic placement of the genus in the subfamily Copelatinae. Bayesian Inference analyses unambiguously indicate that *Rugosus* is nested within *Aglymbus* Sharp, 1880. As a result, *Rugosus* is synonymized with *Aglymbus*. Based on these results and on recent collecting data, the distribution of *Aglymbus* now includes Guyana, Suriname, and Venezuela. High-resolution images of the holotype specimens and labels of *Aglymbus emarginatus* (García), **new combination** and *Aglymbus pubis* (García), **new combination** are provided, as well as comments on the habitat of species in the “*A. lepriurii* species-group” of *Aglymbus*.

Key Words: taxonomy, *Aglymbus*, Bayesian inference, Guyana, phylogenetics, Venezuela

The diving beetle subfamily Copelatinae (Coleoptera: Dytiscidae) contains nearly 700 described species and is distributed across all biogeographic regions (Nilsson 2015). Over the last 20 years, there have been several phylogenetic and/or taxonomic changes proposed for copelatines, mostly from the description of four new genera: *Capelatus* Bilton and Turner, 2015 from South Africa; *Madaglymbus* Shaverdo and Balke, 2008 from Madagascar; *Papuadytes* Balke, 1998 from New Guinea; and *Rugosus* García, 2001 from Venezuela. Among these new taxa, *Papuadytes* was placed as a junior synonym of *Exocelina* Broun, 1886 (Nilsson 2007). *Capelatus* was described based on morphological and molecular evidence using a comprehensive set of gene fragments (Bilton *et al.* 2015). *Madaglymbus* also was described based on morphology and mole-

cular phylogenetics (Shaverdo *et al.* 2008). Finally, *Rugosus* from the Neotropics (Fig. 1) originally was described as a member of the tribe Colymbetini within the subfamily Colymbetinae (García 2001) because at that time the subfamily Copelatinae was considered a tribe of Colymbetinae. However, we noticed that some species of the Neotropical genus *Aglymbus* Sharp, 1880 share a similar morphology with deeply sculptured elytra giving a rough aspect to the beetle’s dorsal surface. Such morphological features can be observed, for instance, in *Aglymbus lepriurii* Aubé. Furthermore, subsequent morphological (Miller 2001) and molecular evidence (Balke *et al.* 2004; Ribera *et al.* 2008; Miller and Bergsten 2014) now suggests that Colymbetinae and Copelatinae are very distinct within the Dytiscidae. Since the described species



Fig. 1. Known distribution of the *Rugosus* species-group in South America

of *Rugosus* (García 2001; Fig. 2) have never been included in a phylogenetic reconstruction, the placement of *Rugosus* with respect to other copelatine genera requires verification.

Because some species of *Aglymbus* share a similar morphology of deeply sculptured elytra, giving a rough aspect to the beetle's dorsal surface, we tested the hypothesis that these two genera are synonymous by investigating the phylogenetic relationships of *Rugosus* among other genera of the subfamily Copelatinae. We sequenced a specimen of *Rugosus emarginatus* García as well as a specimen of *Aglymbus leprieurii* Aubé from Guyana. We combined these new data with the comprehensive matrix of Bilton *et al.* (2015) and infer the phylogenetic relationships using Bayesian inference.

#### MATERIAL AND METHODS

**Taxon Sampling and Molecular Biology.** We used the molecular matrix from Bilton *et al.* (2015), which included the eight following gene fragments: mitochondrial *cytochrome c oxidase 1* (*cox1*, 732bp, 3' end of the gene); *cytochrome c oxidase 2* (*cox2*, 552bp); *cytochrome b* (*cob*, 306bp); nuclear *histone 3* (*H3*, 315bp); *histone 4* (*H4*, 156bp); *18S ribosomal DNA* (*18S*, 546bp); *carbamoylphosphate synthase* (*CAD*, 849bp); and *α-spectrin* (*Asp*, 792bp). To test the phylogenetic placement of

*Rugosus* with respect to other copelatine genera, we extracted a specimen of *R. emarginatus* as well as a specimen of *A. leprieurii* (Table 1). Total genomic DNA was extracted from the whole body using a DNeasy kit (Qiagen, Hilden, Germany). Using the PCR protocols of Toussaint *et al.* (2014), we amplified and then sequenced the *CO1* and *H3* gene fragments. The DNA sequences were edited manually in Geneious R8 (Biomatters, www.geneious.com) and aligned using Muscle (Edgar 2004), and the reading frames were checked under Mesquite 3.01 (mesquiteproject.org). The dataset used to infer phylogenetic relationships was later generated under Mesquite. The new sequences were deposited in GenBank (accession Nos. KT719169-KT719172).

**Molecular Phylogenetics.** We used Bayesian inference (BI) to reconstruct the phylogenetic relationships among genera of Copelatinae. The partitions and corresponding optimal models of substitution were searched under PartitionFinder 1.1.1 (Lanfear *et al.* 2012) using the *greedy* algorithm and the *mrBayes* set of models (Table 2). The Akaike Information Criterion corrected (AICc) was used to compare the fit of the different models. The BI analyses were conducted using MrBayes 3.2.2 (Ronquist *et al.* 2012). Two simultaneous and independent runs consisting of eight Metropolis-coupled Markov chain Monte Carlo (MCMC, one cold and seven incrementally heated) running 50 million generations were used, with a tree sampling every 5,000 generations to calculate posterior probabilities (PP). The convergence of the runs was investigated by checking the split frequencies and effective sample size (ESS) of all the parameters, and by plotting the log-likelihood of the samples against the number of generations in Tracer 1.5 (BEAST.bio.ed.ac.uk/Tracer). A value of ESS > 200 was acknowledged as a good indicator of convergence. All trees predating the time needed to reach a log-likelihood plateau were discarded as burn-in, and the remaining samples were used to generate a 50% majority rule consensus tree.

#### RESULTS

The phylogeny resulting from the MrBayes analysis is given in Fig. 3. Overall, the inferred relationships are robust except for five nodes that only have moderate support but are not particularly relevant to this study. We recovered all genera as reciprocally monophyletic with maximal support (PP = 1.00) except *Aglymbus* and *Rugosus*. Both genera are nested in a clade with all nodes highly supported (PP = 1.00). *Rugosus emarginatus* is resolved as sister to the two *A. leprieurii* specimens, and these together are nested inside other *Aglymbus* taxa. Consequently, we synonymize *Rugosus*, **new synonymy**, with *Aglymbus*. The complex *Aglymbus*+*Rugosus* is



Fig. 2. Dorsal habitus and labels of the holotypes of *Rugosus emarginatus* (above) and *Rugosus pubis* (below).

**Table 1.** List of dytiscid specimens used in this study.

Subfamily	Genus	Species	Code	Country
Copelatinae	<i>Agaporomorphus</i>	<i>knischi</i> Zimmerman	MB0751	Peru
Copelatinae	<i>Agaporomorphus</i>	<i>tambopatensis</i> Miller	MB0753	Peru
Copelatinae	<i>Aglymbus</i>	<i>leprieurii</i> (Aubé)	MB0307	French Guyana
Copelatinae	<i>Aglymbus</i>	<i>leprieurii</i> (Aubé)	SLE935	Guyana
Copelatinae	<i>Aglymbus</i>	<i>pilatus</i> Guignot	MB0645	Venezuela
Copelatinae	<i>Aglymbus</i>	sp.	MB0322	Ecuador
Copelatinae	<i>Capelatus</i>	<i>prykei</i> Bilton and Turner	MB3919	South Africa
Copelatinae	<i>Copelatus</i>	<i>assimilis</i> Régimbart	MB3432	Gabon
Copelatinae	<i>Copelatus</i>	<i>bromeliarum</i> (Scott)	MB1250	Trinidad and Tobago
Copelatinae	<i>Copelatus</i>	<i>gardineri</i> Scott	MB3182	Seychelles
Copelatinae	<i>Copelatus</i>	<i>marginatus</i> Sharp	MB3565	New Caledonia
Copelatinae	<i>Copelatus</i>	<i>tenebrosus</i> Régimbart	MB4862	Sulawesi
Copelatinae	<i>Exocelina</i>	<i>cheesmanae</i> (Balfour-Browne)	MB1396	Vanuatu
Copelatinae	<i>Exocelina</i>	<i>danae</i> (Balke)	MB0673	New Guinea
Copelatinae	<i>Exocelina</i>	<i>gapa</i> (Watts)	MB1813	Australia
Copelatinae	<i>Exocelina</i>	<i>novaecaledoniae</i> (Balfour-Browne)	MB0137	New Caledonia
Copelatinae	<i>Exocelina</i>	sp. 1	MB1290	New Guinea
Copelatinae	<i>Exocelina</i>	sp. 2	MB3405	New Guinea
Copelatinae	<i>Lacconectus</i>	<i>atlas</i> Brancucci	MB5602	Laos
Copelatinae	<i>Lacconectus</i>	<i>punctipennis</i> Zimmermann	MB0043	Malaysia
Copelatinae	<i>Lacconectus</i>	<i>sabahensis</i> Brancucci	MB0626	Malaysia
Copelatinae	<i>Liopterus</i>	<i>atriceps</i> (Sharp)	MB0047	Italy
Copelatinae	<i>Liopterus</i>	<i>haemorrhoidalis</i> (Fabricius)	MB0249	Germany
Copelatinae	<i>Madaglymbus</i>	<i>ruthwildae</i> Shaverdo and Balke	MB1244	Madagascar
Copelatinae	<i>Madaglymbus</i>	sp.	MB0009	Madagascar
Copelatinae	<i>Rugosus</i>	<i>emarginatus</i> García	SLE936	Venezuela
Laccophilinae	<i>Laccophilus</i>	sp.	MB4863	Indonesia

found sister (PP = 1.00) to a large clade comprising *Exocelina*, *Capelatus*, and *Liopterus* Dejean.

## DISCUSSION

Our phylogenetic results are in agreement with Bilton *et al.* (2015). We find *Rugosus* nested within

*Aglymbus* with maximal support (Fig. 3). From a morphological standpoint, this result is not surprising as the morphological features found in *Rugosus* (medium size, dark black body with the surface of the elytra densely covered with short microreticulations) are also found in some species of *Aglymbus*, such as its type species, *A. leprieurii*. We here refer to these species as the “*A. leprieurii* species-group” of the genus *Aglymbus*.

In addition to these described taxa, recent field work in the Guiana Shield region of South America has yielded additional undescribed species that can be placed within the “*A. leprieurii* species-group”. A distribution map of all *Rugosus* species-group specimens we have examined is shown in Fig. 1. Nearly all specimens were collected in small to medium-sized forest pools in densely forested lowlands (*e.g.*, Fig. 4).

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**Table 2.** MrBayes partitions and models of substitution selected in PartitionFinder.

Partition	Composition	Model (AICc)
P1	CO1 1 <sup>st</sup>	GTR+Γ+I
P2	CO1 2 <sup>nd</sup> , H4 3 <sup>rd</sup>	HKY+I
P3	CO1 3 <sup>rd</sup> , CO2 3 <sup>rd</sup> , Cytb 3 <sup>rd</sup>	GTR+Γ+I
P4	CO2 1 <sup>st</sup>	GTR+G
P5	CO2 2 <sup>nd</sup> , Cytb 2 <sup>nd</sup>	GTR+Γ+I
P6	Cytb 1 <sup>st</sup>	GTR+G
P7	18S	SYM+Γ+I
P8	ASP 1 <sup>st</sup> , CAD 1 <sup>st</sup>	GTR+G
P9	ASP 2 <sup>nd</sup>	GTR+I
P10	ASP 3 <sup>rd</sup>	HKY+Γ+I
P11	CAD 2 <sup>nd</sup>	GTR+Γ+I
P12	CAD 3 <sup>rd</sup>	HKY+Γ+I
P13	H3 1 <sup>st</sup>	GTR+I
P14	H3 2 <sup>nd</sup> , H4 2 <sup>nd</sup>	JC
P15	H3 3 <sup>rd</sup>	GTR+G
P16	H4 1 <sup>st</sup>	GTR+G

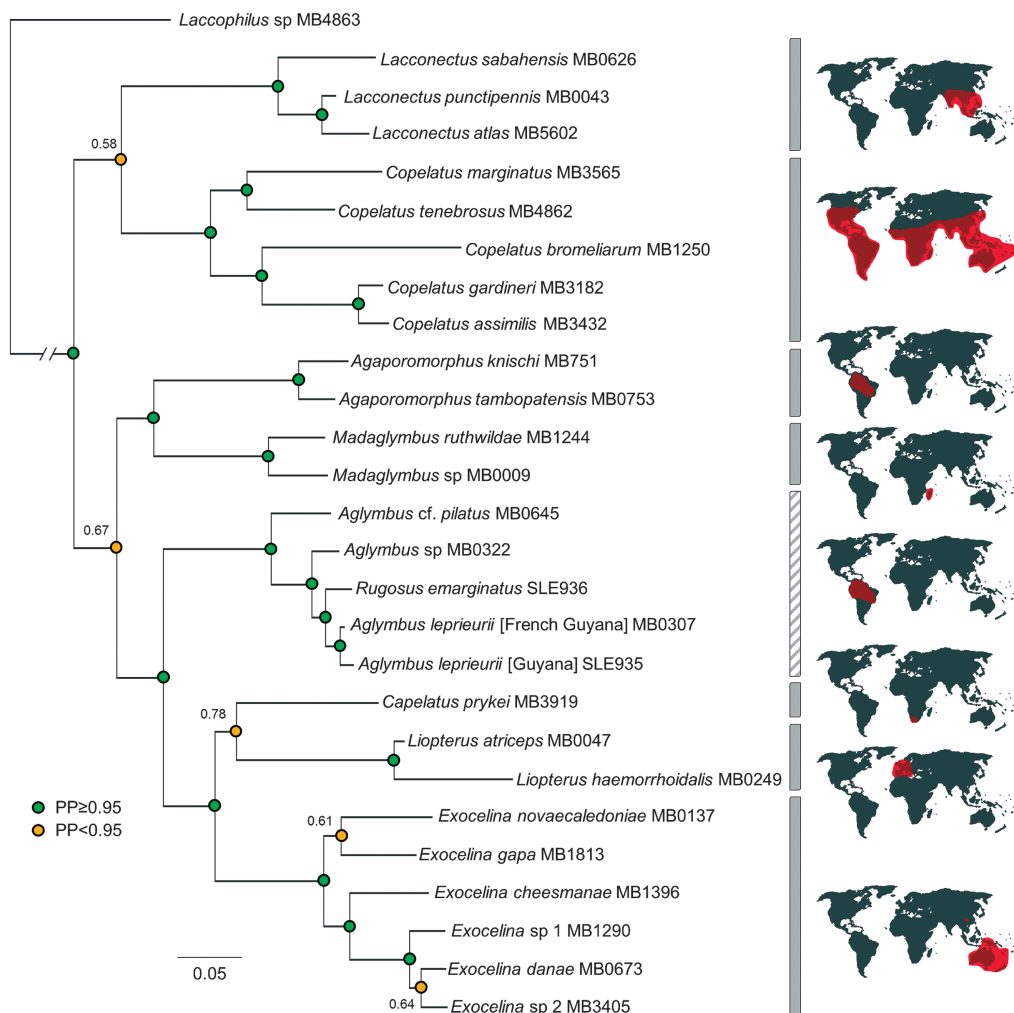


Fig. 3. Molecular phylogeny of the subfamily Copelatinae and placement of *Rugosus*.

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#### REFERENCES CITED

- Balke, M., I. Ribera, and A. P. Vogler. 2004.** MtDNA phylogeny and biogeography of Copelatinae, a highly diverse group of tropical diving beetles (Dytiscidae). *Molecular Phylogenetics and Evolution* 32: 866–880.
- Bilton, D. T., E. F. A. Toussaint, C. Turner, and M. Balke. 2015.** *Capelatus prykei* gen. n., sp. n. (Coleoptera: Dytiscidae: Copelatinae) – a phylogenetically isolated diving beetle from the Western Cape of South Africa. *Systematic Entomology* 40(3): 520–531.
- Edgar, R. C. 2004.** MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797.
- García, M. 2001.** Nuevos Colymbetinae (Coleoptera; Dytiscidae) del sur de Venezuela. *Boletín del Centro de Investigaciones Biológicas* 35(3).
- Lanfear, R., B. Calcott, S. Y. Ho, and S. Guindon. 2012.** PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29(6): 1695–1701.
- Miller, K. B. 2001.** On the phylogeny of the Dytiscidae (Insecta: Coleoptera) with emphasis on the morphology of the female reproductive system. *Insect Systematics & Evolution* 32(1): 45–89.
- Miller, K. B., and J. Bergsten. 2014.** The phylogeny and classification of predaceous diving beetles



**Fig. 4.** Habitat of *Rugosus* species. A) Guyana, Region 8, Ayanganna Airstrip, collecting event GY14-0317-01A, B) Suriname, Sipaliwini District, Kappel Airstrip at base of Tafelberg Tepui, collecting event SR13-0824-03A, C) Suriname, Sipaliwini District, Raleighvallen Nature Reserve, collecting event SR12-0727-01A, D) Guyana, Region 8, Ayanganna Airstrip, collecting event GY14-0319-02A.

[pp. 49–172]. *In: Ecology, Systematics, and the Natural History of Predaceous Diving Beetles* (Coleoptera: Dytiscidae). Springer, The Netherlands.

**Nilsson, A. N. 2007.** *Exocelina* Broun, 1886 is the valid name of *Papuadytes* Balke, 1998. *Latissimus* 23: 33–34.

**Nilsson, A. N. 2015.** A world catalogue of the family Dytiscidae, or the diving beetles (Coleoptera, Adepfaga). Version 1.1.2015. [www2.emg.umu.se/projects/biginst/andersn/](http://www2.emg.umu.se/projects/biginst/andersn/) (accessed 8 May 2015).

**Ribera, I., A. P. Vogler, and M. Balke. 2008.** Phylogeny and diversification of diving beetles (Coleoptera: Dytiscidae). *Cladistics* 24(4): 563–590.

**Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012.** MrBayes 3.2: Efficient Bayesian phylogenetic

inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542.

**Shaverdo, H., M. T. Monaghan, D. C. Lees, R. Ranaivosolo, and M. Balke. 2008.** *Madaglymbus*, a new genus of Malagasy endemic diving beetles and description of a highly unusual species based on morphology and DNA sequence data (Dytiscidae: Copelatinae). *Systematics and Biodiversity* 6(1): 43–51.

**Toussaint, E. F. A., R. Hall, M. Monaghan, K. Sagata, S. Ibalim, H. V. Shaverdo, A. P. Vogler, J. Pons, and M. Balke. 2014.** The towering orogeny of New Guinea as a trigger for arthropod megadiversity. *Nature Communications* 5: 5001.

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